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Original Article

Prevalence of *Echinococcus* spp. Infection Using Coproantigen ELISA among Canids of Moghan Plain, Iran

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Abstract

Background: Echinococcosis is one of the most important helminthic zoonotic diseases in Iran. Intestinal Scraping Technique (IST), the traditional method for diagnosis of infection in definitive hosts, has many disadvantages including low susceptibility and being expensive, hazardous and laborious. Detection of coproantigens in fecal samples by enzyme-linked immunosorbent assay (CA-ELISA) is known as a useful tool for intravital mass-screening of definitive host populations. This study was performed to determine the prevalence of *Echinococcus* spp. infection among canids in Moghan plain, the only area in Iran known as endemic for *E. multilocularis*.

Methods: One hundred thirty eight fecal samples were collected from red foxes and domestic dogs in three counties of Moghan plain namely Pars Abad, Bileh Savar and Germi. The samples were examined using an ELISA, designed for the detection of *Echinococcus*-specific coproantigen and the formalin-ether concentration method as well.

Results: Totally, out of 138 fecal samples, 27 (21.6%) turned positive for *Echinococcus* coproantigen. coproantigen was detected in 16.7% and 27.1% of red foxes and domestic dogs, respectively. Formalin-ether concentration method revealed that 43 (31.2%) of samples harbored at least one parasitic helminth, but *Taenia* eggs were detected only in 3 fecal samples. Since coproantigen presence reflects current intestinal infection with adult worms, CA-ELISA can be regarded as one of the most useful immunological tools for diagnosis of *Echinococcus* infection. Besides, the high susceptibility, less expense and rapidity make it the method of choice for epidemiological surveys in endemic areas of Iran.

Keywords: *Coproantigen, Echinococcosis,* Echinococcus, *CA-ELISA, Canids, Iran*

Introduction

Echinococcosis is a zoonotic infection disease caused by adult or larval (metacestode) stages of cestodes belonging to the genus *Echinococcus* (family: *Taeniidae*). The parasites are perpetuated in life cycles with carnivores as definitive hosts, which harbor the adult egg-producing worm in the intestine, and intermediate host animals, in which the infective metacestode stage develops after per oral infection with eggs. Metacestodes may incidentally also develop in humans leading to various forms of echinococcosis (1). This helminthic disease is prevalent throughout Iran. Adult *Echinococcus granulosus* worms have been detected in various carnivores such as stray and farm dogs, red foxes, golden jackals and wolves from many provinces (2- 4) including rural and urban areas of Kerman (5), Khuzestan (6), Fars (7), Tehran (8), Kurdestan (9), Mashhad in Khorasan (10) and Kashan region in Isfahan (11) and western provinces of Iran (12).

In Iran, being recognized as an endemic country for Alveolar Echinococcusis (AE) (13), the first evidence of *Echinococcus multilocularis* infection in canids was described in 1971, in which 3 out of 30 red foxes (10%) were found to be infected with cestode of *E. multilocularis* (14, 15). The second report on 130 wild and domestic carnivores from Ardebil province in northwestern Iran in 1992 showed that 22.9% of red foxes

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(*Vulpes vulpes*) and 16% of jackals (*Canis aureus*) were infected with adult stages of *E. multilocularis* (16, 17). In these two reports, the diagnosis was based on morphological features of *Echinococcus* adult worms at necropsy.

Since the eggs of *Echinococcus* and *Taenia* species are morphologically indistinguishable, diagnosis of *Echinococcus* infection in fecal samples of definitive hosts is difficult (18). Besides, the characteristic small segments of *Echinococcus* worms may be absent in the feces or be easily overlooked (1). By the end of the 1980's the only reliable technique for diagnosis of intestinal *Echinococcus* infection in definitive hosts was intestinal scraping technique (IST) at necropsy and examination of scraped materials under stereoscope. This technique, with maximum sensitivity of 78% in optimal condition (19) relies on inspection of the dead animal's intestine and visual identification of the worms according to their morphological features (18). IST is considered as an expensive, biohazardous and laborious diagnostic method and is not recommendable for examination of domestic animals. In addition, dogs and red foxes are known to be susceptible to both species of *E. granulosus* and *E. multilocularis* and in some regions they might even be infected with both *Echinococcus* species (19). Recently, two new techniques based on detection of Copro-DNA by PCR and Coproantigen by enzyme-linked immunosorbent assays (CA-ELISA) were introduced for intravital diagnosis of intestinal parasitic infections of carnivores.

To date, most of studies on prevalence of helminthic infections of carnivores in Iran were merely based on the traditional method of IST. Recently, Siavashi et al. (20) used CA-ELISA for detection of canine echinococcosis in three provinces of Iran. They reported the specificity and sensitivity of CA-ELISA to be 74% and 72%, respectively.

The main aim of this study was to determine the prevalence of *Echinococcus* spp. infection among canids using CA-ELISA technique in Moghan plain, northwestern Iran, the only area in Iran known as endemic for *E. multilocularis* infection.

Material and Methods

Study area

This study was performed in the Moghan Plain (local name: Dasht-e-Moghan) in the northwestern province of Ardebil, Iran (Fig. 1). The area comprises 3 counties including Pars Abad, Bileh Savar and Germi covering an area of nearly 5245 Km^2 with a total population of approximately 310,000. The study area covered the low landing areas with altitude of 32 m up to plains of 1023 m high. The longitudes and latitudes ranges were approximately 46°52'53″E- 48°21'30″ E and 39° 0' 0″N -39°36'20″N, respectively with average annual precipitation of 222.76 mm. The area is bordered with Azerbaijan Republic to the north and east and the inhabitants are mainly of Azeri ethnic group, mostly practice farming and Stockbreeding.

Samples

One hundred thirty eight fecal fecal samples including 59 from domestic dogs (*Canis lupus* f. *familiaris*) and 79 from red foxes (*Vulpes vulpes*) (74 rectum-derived from necropsied foxes and 5 from environmentally deposited feces around red fox dens) were collected and examined. As fecal samples might contain eggs or proglottides of *E. granulosus* and *E. multilocularis*, all samples were frozen at least for one week in -70 °C and then kept at -20 °C until used. In addition to CA-ELISA, All samples were concentrated by formalin-ether method and examined microscopically for ova and larvae and cysts of parasites.

CA-ELISA

Detection of coproantigen was performed using a commercially available rabbit polyclonal antibodies based ELISA kit (Chekit Echinotest Monophasic; Bommeli, Liebefeld-Bern, Switzerland), designed for the detection of *E. granulosus* and *E. multilocularis* coproantigens in dogs, foxes and cats. Briefly, 1 g of fecal sample was suspended in 4 volumes of Chekit-Echinotest sample diluents and completely mixed using a shaker. The suspensions were centrifuged at 3000 g for 10 min at room temperature and 2 ml of supernatants were collected and stored in -20 °C until used for CA-ELISA. The procedure for CA-ELISA

was according to the manufacturer's instructions. All preparations were red by a photo spectrometer at a wavelength of 450 nm. Test results were calculated according to formula supplied by kit manufacturer and the values of <30%, 30-40% and >40% were interpreted as negative, ambiguous and positive, respectively. All ambiguous samples were double checked.

Results

Out of 138 fecal samples, 27(19.6%) were shown to be positive for coproantigen, which included 13.9% and 27.1% of red foxes and domestic dogs, respectively (Table 1). The difference between the percentage of coproantigen positive red foxes and domestic dogs were statistically significant (Chi-square test; $\chi^2 = 10.965$; df= 2; $P < 0.005$). There was no significant statistical relation between CA-ELISA results and the location or time of sampling.

Formaline-ether concentration method revealed *Taenia*-like eggs in 3 (2.2%) samples. 43 specimen (32.6%) were found to be infected with at least one parasitic helminth egg and some of them harbored more than one helminth egg (Table 2). There was no significant difference between foxes (26.6%) and dogs (37.3%) regarding overall infection with parasitic worms. There was no relationship between the time of sampling and the results of the method. However, the prevalence of helminthic infections in red foxes from Germi county was significantly lower than those from Bileh Savar and Pars Abad (P <0.001).

Table 1: Frequency table of CA-ELISA Results according to host species

Host Species	CA-ELISA Results			Total
	Negative	Ambiguous	Positive	
Foxes	65(82.3%)	3(3.8%)	11(13.9%)	79
Dogs	34(57.6%)	9(15.3%)	16(27.1%)	59
Total	99(71.7%)	12(8.7%)	27(19.6%)	138

Table 2: Prevalence of parasitic helminthes in red foxes and dogs of Moghan plain by formalin-ether concentration method

Helminths	Number	Percent
Toxascaris spp.	21	15.2
Toxocara canis	16	11.6
Rictolaria spp.	12	8.7
Acanthacephala spp.	10	7.2
Trichuris vulpis	6	4.3
Taenia spp.	3	2.2
Mesocestoides spp.	2	1.4
Hookworms	1	0.7
Capillaria spp.	1	0.7
Fasciola spp.	1	0.7

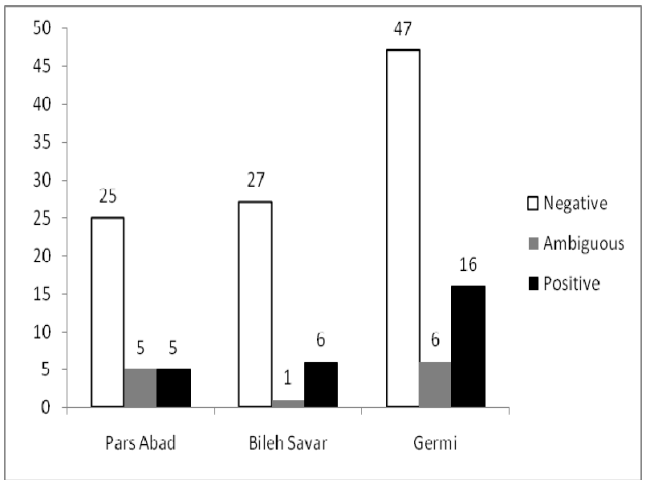

Fig. 2: Frequency of various results of CA-ELISA according to the location of sampling

Fig. 1: Moghan Plain, comprising three counties of Pars Abad, Bileh Savar and Germi in northern parts of Ardebil province, northwestern Iran

Discussion

Echinococcosis is a zoonotic parasitic disease of global concern caused by adult or larval stages of cestodes belonging to the genus *Echinococcus*. In natural life cycle of infection, Carnivores and mammals play the role of definitive and intermediate hosts, respectively. *E. granulosus* and *E. multilocularis* are the two major species of medical importance causing cystic echinococcosis and alveolar echinococcosis, respectively. They are both serious life-threatening diseases, especially the latter one, which is of high fatality rate and poor prognosis without careful clinical management (19). A good knowledge of transmission biology of the helminth is required to predict the infection risk and to adopt proper control measures. Until recently, the methods commonly used for surveys of the *Echinococcus* infection in canids populations included arecoline purging, intestinal scraping technique (IST), sedimentation and counting technique (SCT) but nowadays, the methods of coproantigen detection by ELISA (CA-ELISA) and detection of Echinococcus DNA in stools by PCR have been introduced. There are some reports on detection of *Echinococcus* coproantigens with *Echinococcus*-specific ELISA in various epidemiological studies in many countries including Swiss (21, 22), France (23), Kazakhstan (24) Slovakia (25), Poland (26, 25), Norway (27) and Japan (28-33). This method has shown to be a useful tool for both post mortem and intravital diagnosis of *Echinococcus* in definitive hosts during prepatent as well as patent periods of infection. It has also allowed detection of the infection in field-collected fecal samples in the relevant studies (34). Besides, this method is very fast, allowing examination of about 200 samples per day (22). Therefore this method is considered as a suitable tool for mass screening of definitive host populations.

Different types of CA-ELISA's have shown rather high sensitivities ranging between 84 to 95%, and very high specificities of above 96% (as far as non-*Echinococcus* cestodes and other parasites are concerned). Some studies have shown that sensitivity of this method is increased with worm burden i.e. the number of adult parasites (22), however in Siavshi et al. (20) study, no significance difference between the groups of high and low intensities of infection was observed. Siavashi et al. (20) and Christofi et al. (35) showed that CA-ELISA in a region with low prevalence rate of canine echinococcosis is of low sensitivity and positive predictive value and high specificity and negative predictive value.

Various studies on helminthic infections of carnivores in Iran including Moghan plain using IST at necropsy have shown high prevalence of echinococcosis with 5 to 49% of stray or sheep dogs harboring adult *E. granulosus* (4). IST revealed

that 10 to 25% of red foxes in Moghan plain were infected with *E. multilocularis* (36, 17). Also, in Siavashi et al. (20) study on various carnivores from Hamadan, Azarbaijan and Tehran provinces using CA-ELISA, the prevalence of *Echinococcus* infection was 43.1%. So, being sure of high rates of *Echinococcus* infection in the area, we applied CA-ELISA in absence of classical method of IST. However the results of this survey were roughly consistent to the result of other studies. Lower prevalence of *Echinococcus* infection in our study in comparison to former ones seems to be due to intensive economic and social alterations including population growth and development of villages and cities which has resulted to major ecological changes in the region such as shrinking the territories of wild carnivores. This change seems to be the most important cause of the decrease the decrease in *Echinococcus* infection rate in the area.

All available commercial coproantigen-ELISA's are based on polyclonal antibodies and are genus-specific. They cannot differentiate between *E. granulosus* and *E. multilocularis*. Consequently there would be cross-reactivity in areas where two species occur sympatrically in definitive hosts. A newly developed rapid immunochromatography method (37) and a coproantigen-based ELISA (28), that is to be commercialized soon, (personal communication with Professor Masao Kamiya, Rakuno Gakuen University, Hokkaido, Japan) used monoclonal antibodies against *E. multilocularis* antigen Em9. However, none of them were *E. multilocularis*-species-specific. Hence, in order to make the diagnosis at species level, it is recommended to examine coproantigen-positive samples by PCR assay (1).

This study also demonstrated the high rates of other Zoonotic helminthic infections in red foxes and domestic dogs, especially geohelminths like *Toxocara canis*, *Toxascaris spp* and hookworms, the causative agents of Visceral Larva Migran (VLM) and Cutaneous larva migrans (CLM) (38). The presence of these helminthes indicates a high infestation of rural environment which could be considered as a health concern in the study area.

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The authors declare that they have no conflict of interests.

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